



(19)

Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

EP 0 882 454 A2

(12)

EUROPEAN PATENT APPLICATION

(43) Date of publication:
09.12.1998 Bulletin 1998/50

(51) Int. Cl. 6: A61K 49/00

(21) Application number: 98106720.0

(22) Date of filing: 14.04.1998

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: 18.04.1997 IT MI970929

(71) Applicants:
• BRACCO S.p.A.
20134 Milano (IT)
Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE

• DIBRA S.p.A.
20122 Milano (IT)
Designated Contracting States:
GB IE

(72) Inventors:
• Malsano, Federico
20134 Milano (IT)
• Gozzini, Lulgia
20134 Milano (IT)

(74) Representative:
Minoja, Fabrizio, Dr.
Blanchetti Bracco Minoja S.r.l.
Via Rossini, 8
20122 Milano (IT)

(54) A process for the conjugation of chelants with molecules containing amino groups

(57) A process for the preparation of macromolecular chelants characterized by a high content in chelant groups per macromolecule.

EP 0 882 454 A2

BEST AVAILABLE COPY

Description

The present invention relates to a method for the preparation of macromolecular chelants characterized by a high content in chelant groups per macromolecule. After complexation with suitable metal ions, said compounds can be used, as such or in combination or formulation with other components, in diagnostic imaging as general or specific contrast agents for a certain tissue, organ or body district.

In diagnostic imaging with nuclear magnetic resonance (MRI), high amounts of paramagnetic metal ions, such as gadolinium, have to be administered in order to reach and keep for some time a high signal in specific body districts. The high toxicity of these metal ions can be reduced to a great extent by complexation with suitable chelants, such as diethylenetriaminopentaacetic acid (DTPA); moreover, a sufficiently long-lasting signal is usually obtained by binding these complexes to a macromolecule, for example a protein such as serum albumin (Ogan M.D. et al., Invest. Radiol. 22, 665-671, 1987). The preparation of these conjugates of DTPA with macromolecules is usually carried out by reacting DTPA dianhydride, a commercially available product, with suitable reactive functional groups of the selected macromolecule, such as the amino groups. This method, however, suffers from a number of drawbacks, in that the reaction is not specific and both inter- and intra-molecular bridges (cross-linking) are introduced on the macromolecule, due to the double functionality of the used reactive (Maisano F. et al., Bioconj. Chem. 3, 212-217, 1992). The higher the molar ratio of DTPA dianhydride to macromolecule reactive groups in the conjugation reaction, the more serious said drawbacks. On the other hand, high molar ratios are essential to obtain appreciable conjugation levels, due to the instability of the anhydride in aqueous medium.

In order to overcome these problems, methods have been proposed requiring a complex, expensive synthesis of chelants monofunctionalized with groups specifically reacting with the amino groups of the macromolecule, such as the isothiocyanate group (see for ex. Brechbiel M.W. et al. Inorg. Chem. 25, 2772-2781, 1986; Westerberg D.A. et al., J. Med. Chem. 32, 236-243, 1989). Alternatively, methods envisaging a pre-activation of the chelant in the form of N-succinimido ester and the subsequent reaction with the amino groups of the macromolecule have also been described (Buckley R.G. and Searle F. FEBS Lett. 166, 202-204, 1984; Paxton R.J. et al. Cancer Res. 45, 5694-5699, 1985). The latter alternative, although apparently promising, has not have an adequate development for introducing a high number of chelant groups, as it is necessary in MRI imaging. Said method was in fact described and developed only to conjugate chelates of radioactive metals with antibodies, in view of their use in scintigraphy. This diagnostic technique requires of course extremely low dosages and therefore a great number of chelants conjugated with the macromolecule are of no interest to the researcher of the field.

The present invention overcomes these problems, in that it allows to bind a high number of chelants to the macromolecule, avoiding at the same time non specific reactions, as well as any inter- or intra- molecular cross-linkings. The method object of the present invention is based first on a preactivation of the chelant to conjugate with the macromolecule, transforming it into a suitable reactive derivative, such as, preferably, a N-succinimido ester [analogously to what already known from the prior art]. Nevertheless, contrary to the already described methods, this method provides exceedingly high conjugation levels, thanks to the subsequent original multistep procedure which, besides obviating all the known synthetic difficulties, also makes the application on the industrial scale possible. The preactivation of the chelant as N-succinimido ester is not, in fact, capable alone of giving the necessarily high conjugation levels, even in the presence of a strong molar excess of reactive ester to available amino groups, mainly for two reasons:

- 40 a) the chelant groups conjugated with the macromolecule exert an electrostatic repulsion towards other chelant molecules, thereby decreasing the reactivity of the still free, spatially close amino groups; moreover
- b) in the conjugation conditions, the reactive ester undergoes a simultaneous hydrolysis reaction which inhibits its reactivity.

45 As a consequence, even strong excesses of reactive ester, longer reaction times or more drastic reaction conditions provide no improvements in the total yield of the process. On the contrary, the formation of inner salt bridges between free amino groups and molecules of already conjugated chelant, worsens the situation.

It has now unexpectedly been found that when, after a first conjugation step effected with a reactive ester molar excess and in mild reaction conditions (not high temperature and reaction time), the introduced chelating groups are complexed with suitable metal cations, including radioisotopic metals, their inhibiting action on the reactivity of spatially close amino groups markedly decreases. At this point, a second conjugation reaction allows to bind other chelant molecules also to those amino groups which could not react before.

It is therefore object of the present invention a process for the preparation of conjugates between polyaminopolycarboxylic chelants and molecules containing amino groups, substantially comprising the following steps:

- formation of a reactive ester, such as, preferably, a N-succinimido ester of the desired chelant, by condensation of the chelant with N-hydroxysuccinimide (NHS), in the presence of a suitable dehydrating agent, such as carbodiim-

ide,

- reaction of this ester with the molecule containing the amino groups, being the ester in molar excess to the amino groups of said molecule,
- purification of the resulting conjugate from the excess chelant and subsequent complexation of the conjugate with a metal ion,
- second conjugation reaction between the complexed conjugate and the chelant N-succinimido ester, being the ester in molar excess to the residual amino groups,
- purification of the final conjugate from the excess chelant and subsequent second complexation of the residual chelants introduced with a metal ion.

10

A particularly preferred embodiment of the process according to the present invention comprises:

- preparation of the N-succinimido ester of the polyaminopolycarboxylic chelant, preferably DTPA or DOTA, by reaction with NHS, in the presence of dicyclohexylcarbodiimide in an aprotic solvent selected from acetonitrile, dimethylsulfoxide, dimethylformamide and optionally in the presence of an organic base,
- subsequent concentration of said ester by evaporation under vacuum of most solvent, i.e. about 90%;
- gradual addition of the ester concentrated solution to an aqueous solution of the amino group containing-molecule, keeping pH between 6 and 10 by the simultaneous addition of a base, preferably NaOH; when the ester amount, calculated on the basis of the starting chelant, is in a 5 - 250, preferably 6 - 100, molar excess, to the amino groups present in the molecule and the reaction time ranges from 1 to 48 hours from the end of the additions of the ester, at a temperature from 15 to 40°C,
- purification of the resulting conjugate from by-products, impurities and excess reagents by membrane (dialysis or ultrafiltration), or chromatographic (ion exchange or steric exclusion chromatographies) processes, or by a combination thereof,
- complexation of the conjugate with a metal ion or a salt thereof and optional subsequent removal of the excess metal,
- a second conjugation reaction between the still free amino groups of the complexed conjugate with a molar excess of the N-succinimido ester chelant, in the conditions described above,
- second purification of the conjugate from the reaction by-products by means of membrane or chromatographic processes,
- complexation of the final conjugate with a metal ion and optional subsequent purification treatments to remove the excess metal and/or to improve the homogeneity of the compound.

Polyamine macromolecules susceptible of conjugation according to the above described method comprise, for example, bovine serum albumin, insulin, cytochrome C, myoglobin, dendrimers, polylysines and the like. Among the most suitable metal ions, Fe^{2+} , Fe^{3+} , Cu^{2+} , Cr^{3+} , Gd^{3+} , Eu^{3+} , Dy^{3+} , La^{3+} , Yb^{3+} or Mn^{2+} can be cited, whereas the radioisotopic ions can be ^{51}Cr , ^{67}Ga , ^{68}Ga , ^{111}In , ^{99m}Tc , ^{140}La , ^{175}Yb , ^{153}Sm , ^{166}Ho , ^{90}Y , ^{149}Pm , ^{177}Lu , ^{47}Sc , ^{142}Pr , ^{159}Gd , ^{212}Bi .

The method of the invention provides chelate/macromolecule conjugation percentages exceedingly higher than those of the teachings of the prior art. It is moreover possible to obtain metal complex conjugates characterized by a chelant/amino groups total substitution degree of at least 50% and, depending on the macromolecule used, even higher than 60/70%. Furthermore, non specific and cross-linking reactions are prevented. The purification of the final mixture from the undesired impurities (in this case only the excess chelant and N-hydroxysuccinimide) is therefore easier, as exemplified in the following Experimental section.

45

Example 1

Preparation of Gd-DTPA - bovine serum albumin conjugate

50 A solution of 40 g DTPA (0.102 mol) in 1.2 l of dry dimethylsulfoxide (DMSO) is prepared by heating and stirring, then it is cooled at room temperature and added with a solution of 11.73 g NHS (0.102 mol) in 300 ml DMSO, then, drop by drop, with a solution of 19.6 g of N,N'-dicyclohexylcarbodiimide (0.097 mol) in 400 ml DMSO. The mixture is stirred for 16 hours, then filtered and the filtrate is concentrated by evaporation at 50°C and 5 Pa to a thick oil of an about 160 ml volume.

55 This oil is added in portions to a solution of 1 g of bovine serum albumin (BSA; 15 μ mol, 60 amino groups/molecule) in 1 l of 0.1 M borate buffer pH 8, 0.1 M NaCl, keeping pH at 8 by the simultaneous addition of 2 N NaOH. At the end of the additions, the mixture is stirred for 16 hours, then filtered and purified from by-products and excess reagents by chromatographic desalting on a Sephadex G-25 column (8.9 x 50 cm). The protein solution is then concentrated by

ultrafiltration (S1Y30 cartridge, Amicon) to a volume of 100 ml. This solution is added with 10 ml of a 0.6 M solution of the complex of gadolinium with nitrilotriacetic acid at pH 6 and equilibrated for 1 hour, after that it is diluted to 2 l with borate buffer pH 8 and concentrated again to 100 ml by ultrafiltration.

5 The resulting compound contains about 25 Gd-DTPA residues per albumin molecule. To further increase this ratio, the solution of the complexed conjugate is treated again with the N-succinimido ester as described above, and subjected again to the described purification and complexation procedures. After the complexation, the compound is finally purified by steric exclusion chromatography on a Sephadex S-200 HR column (65 cm x 9 cm diam.) eluting with 0.15 M NaCl. This chromatography also purifies the compound from the dimer and aggregates percentages originally present in the commercial BSA, thereby providing a conjugate with 45 Gd-DTPA residues per protein molecule (75% of 10 the 60 amino groups of BSA), which is chromatographically homogeneous and free from cross-linking and from free Gd-DTPA.

15 The determination of the substitution degree was effected referring the gadolinium concentration to that of the protein, determined by X-ray fluorescence (XRF) and spectrophotometric absorption at 280 nm ($e_{280\text{ nm}} = 0.66 \text{ L} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$), respectively.

15

Table 1

Characteristics of the compounds described in example 1.			
Compound	Gd mol/mol BSA	Substitution degree	% aggregates
Commercial BSA	-	0	20 - 25%
Gd-DTPA-BSA (1 conjug.)	25	42%	20 - 25%
Gd-DTPA-BSA (2 conjug.+purif.)	45	75%	0

25

Example 2

Preparation of conjugates of other proteins with Gd-DTPA

30

According to the same procedure, Gd-DTPA was conjugated with porcine insulin, equine cytochrome C, equine myoglobin, to obtain conjugates containing 3, 13 and 12 Gd-DTPA residues/protein respectively, corresponding to 100%, 68% and 60% amino groups substitution (see Table 2).

35

Table 2

Characteristics of the compounds described in example 2			
Compound	Starting amino groups	Gd mol/mol BSA	Substitution degree
Gd-DTPA-insulin	3	3	100%
Gd-DTPA-cytochrome C	19	13	68%
Gd-DTPA-myoglobin	20	12	60%

45

Claims

1. A process for the conjugation of polyaminopolycarboxylic chelants with the amino groups of molecules/macromolecules, which essentially comprises the following steps:

50

- activation of said chelants, by formation of a reactive ester, such as a N-succinimido ester of the same;
- conjugation of said reactive esters with the amino groups of the molecules to functionalize;
- removal of the unreacted chelant and formation of the metal complex of the conjugate from step b);
- reaction of said metal complex with the reactive chelants of step a);
- removal of the unreacted chelant and second complexation with metal ions, analogously to step c).

55

2. A process according to claim 1, in which step a) is carried out by condensation of the polyaminopolycarboxylic chelant with N-hydroxysuccinimide in the presence of a dehydrating agent in an aprotic solvent, optionally in the

presence of an organic base.

3. A process according to claims 1-2, in which step b) is carried out in the presence of a reactive chelant molar excess to the number of amino groups of the molecules to functionalize, in aqueous medium and at pH from 6 to 10.
- 5 4. A process according to claims 1-3, in which step c) is carried out first removing impurities, by-products and excess reagents by membrane and/or chromatographic processes, then reacting the resulting conjugate with a metal ion or a salt thereof.
- 10 5. A process according to claims 1-4, in which step d) is carried out reacting the still free amino groups of the complexed conjugate with a reactive chelant molar excess, in the conditions above described.
6. A process according to claims 1-5, in which step e) is carried out analogously to what described in claim 4.
- 15 7. A process according to claim 2, in which the dehydrating agent is dicyclohexylcarbodiimide and the aprotic solvent is selected from the group of acetonitrile, dimethylsulfoxide, dimethylformamide.
8. A process according to claim 3, in which the chelant reactive ester is in a 5 - 250 molar excess to the amino groups, pH is kept between 6 and 10 by addition of NaOH, reaction time ranges from 1 h to 48 h from the end of the ester addition and temperature is kept from 15° to 40°C.
- 20 9. A process according to claim 4, in which impurities, by-products and excess reagents are removed by dialysis or ultrafiltration, or by ion exchange or steric exclusion chromatographies, or by a combination of said methods.
- 25 10. A process according to the above claims, in which the polyaminopolycarboxylic chelant is selected from DTPA and DOTA.
11. A process according to the above claims, in which the polyamine macromolecule is selected from bovine serum albumin, insulin, cytochrome C, myoglobin, dendrimers and polylysines.
- 30 12. A process according to the above claims, in which Cr(3+), Gd(3+), Eu(3+), Dy(3+), La(3+), Yb(3+) or Mn(2+) or from the ions of the following radioisotopes: ⁵¹Cr, ⁶⁷Ga, ⁶⁸Ga, ¹¹¹In, ^{99m}Tc, ¹⁴⁰La, ¹⁷⁵Yb, ¹⁵³Sm, ¹⁶⁶Ho, ⁹⁰Y, ¹⁴⁹Pm, ¹⁷⁷Lu, ⁴⁷Sc, ¹⁴²Pr, ¹⁵⁹Gd, ²¹²Bi.
- 35 13. Metal complexed conjugates with a high chelant residue/amino groups ratio, obtained according to the process of the above claims.
14. Metal complexed conjugates according to claim 13, characterized by a chelant residue/amino groups total substitution degree of at least 50%.
- 40 15. Diagnostic pharmaceutical compositions containing at least one of the complex conjugates according to the above claims as contrast agents.

45

50

55



(19) Europäisches Patentamt

European Patent Office

Office européen des brevets



(11) EP 0 882 454 A3

(12)

EUROPEAN PATENT APPLICATION

(88) Date of publication A3:
08.11.2000 Bulletin 2000/45

(51) Int. Cl. 7: A61K 49/00

(43) Date of publication A2:
09.12.1998 Bulletin 1998/50

(21) Application number: 98106720.0

(22) Date of filing: 14.04.1998

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: 18.04.1997 IT MI970929

(71) Applicants:

• BRACCO S.p.A.
20134 Milano (IT)

Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE

• DIBRA S.p.A.
20122 Milano (IT)
Designated Contracting States:
GB IE

(72) Inventors:
• Maisano, Federico
20134 Milano (IT)
• Gozzini, Lulgia
20134 Milano (IT)

(74) Representative:
Minoja, Fabrizio, Dr.
Blanchetti Bracco Minoja S.r.l.
Via Rossini, 8
20122 Milano (IT)

(54) A process for the conjugation of chelants with molecules containing amino groups

(57) A process for the preparation of macromolecular chelants characterized by a high content in chelant groups per macromolecule.

EP 0 882 454 A3



European Patent
Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention EP 98 10 6720
shall be considered, for the purposes of subsequent
proceedings, as the European search report

DOCUMENTS CONSIDERED TO BE RELEVANT					
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (B6CL6)		
Y	US 5 554 748 A (WATSON ALAN D ET AL) 10 September 1996 (1996-09-10) * abstract * * column 10, line 54 - column 11, line 9 * * examples 1-19 * * claims 1-5 * ---	1-12,15	A61K49/00		
Y	EP 0 315 220 A (GANSOW OTTO A) 10 May 1989 (1989-05-10) * abstract * * column 1, line 39 - column 3, line 14 * * column 4, line 48 - column 8, line 39 * --- -/-	1-12,15			
INCOMPLETE SEARCH					
The Search Division considers that the present application, or one or more of its claims, does/does not comply with the EPO to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.					
Claims searched completely :					
Claims searched incompletely :					
Claims not searched :					
Reason for the initiation of the search:					
see sheet C					
Place of search	Date of completion of the search	Examiner			
MUNICH	8 September 2000	Taylor, G.M.			
CATEGORY OF CITED DOCUMENTS					
X : particularly relevant if taken alone	T : theory or principle underlying the invention				
Y : particularly relevant if combined with another document of the same category	E : earlier patent document, but published on, or after the filing date				
A : technological background	D : document cited in the application				
C : non-patent disclosure	L : document cited for other reasons				
P : intermediate document	B : member of the same patent family, corresponding document				



PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 98 10 6720

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (InCLUS)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	TECHNICAL FIELDS SEARCHED (InCLUS)
D,Y	<p>PAXTON R J ET AL: "HIGH-SPECIFIC-ACTIVITY 111IN-LABELED ANTICARCINOEMBRYONIC ANTIGEN MONOCLONAL ANTIBODY: IMPROVED METHOD FOR THE SYNTHESIS OF DIETHYLENETRIAMINEPENTAACETIC ACID CONJUGATES" CANCER RESEARCH, US, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, vol. 45, no. 11, 1 November 1985 (1985-11-01), pages 5694-5699, XP9800569506 ISSN: 0008-5472 * abstract * * Materials and Methods *</p>	1-12,15	
D,Y	<p>R.G. BUCKLEY AND F. SEARLE: "An efficient method for labelling antibodies with 111-In" FEBS LETTERS, vol. 166, no. 1, 1984, pages 282-284, XP880918645 * abstract * * Materials and Methods *</p>	1-12,15	
P,A	<p>WO 97 32862 A (BRACCO SPA ;DIBRA SPA (IT); GOZZINI LUIGIA (IT); MAISANO FEDERICO) 12 September 1997 (1997-09-12) * abstract * * page 1, line 1 - line 27 * * claims 1-23 *</p>	1-12,15	



Claim(s) searched completely:
10,11

Claim(s) searched incompletely:
1-9,12,15

Claim(s) not searched:
13,14

Reason for the limitation of the search:

Present claims 1-9 and 12-15 relate to an extremely large number of possible products. Support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC is to be found, however, for only a very small proportion of the products claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the products as further defined in the subject-matter of claims 10 and 11, namely conjugates of a polyaminopolycarboxylic acid selected from those defined in claim 10, and polyamine macromolecules selected from those defined in claim 11.

Claims 13 and 14 relate to a product defined by reference to a desirable characteristic or property, namely conjugates with a high chelant residue/ amino groups ratio.

The claims cover all products having this characteristic or property, whereas the application provides support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Independent of the above reasoning, the claims also lack clarity (Article 84 EPC). An attempt is made to define the products by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. In particular, the claims relate to the underlying technical problem as outlined in the description on p.1, lines 1-4, p.2, lines 5-7 and p.2, line 31 to p.3, line 4.

In view of this, and the restriction of the search of the other claims, claims 13 and 14 have not been searched.

ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.

EP 98 18 6728

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EPO file on. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

08-09-2000

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US 5554748 A	10-09-1996	AT 139798 T		15-07-1996
		AT 150847 T		15-03-1997
		AU 656304 B		02-02-1995
		AU 5423598 A		05-11-1998
		CA 2051648 A		08-10-1990
		DE 69027603 D		01-08-1996
		DE 69027603 T		05-12-1996
		DE 69830186 D		17-04-1997
		DE 69830186 T		19-06-1997
		WO 9012050 A		18-10-1990
		EP 0474642 A		18-03-1992
		EP 0481526 A		22-04-1992
		ES 2088428 T		16-08-1996
		ES 2098299 T		01-05-1997
		HK 1003577 A		30-10-1998
		HK 1003578 A		30-10-1998
		HU 60277 A		28-08-1992
		IE 74852 B		13-08-1997
		JP 4504436 T		06-08-1992
		NO 178866 B		11-03-1996
		US 5364613 A		15-11-1994
		US 5914095 A		22-06-1999
<hr/>				
EP 0315220 A	10-05-1989	US 4824986 A		25-04-1989
		AU 4932490 A		07-06-1990
		AU 596590 B		10-05-1990
		AU 5780086 A		18-11-1986
		BR 8606644 A		04-08-1987
		CN 86103488 A		27-05-1987
		DK 627286 A		26-02-1987
		EP 0220279 A		06-05-1987
		EP 0355860 A		28-02-1990
		ES 554387 D		01-07-1987
		ES 8706965 A		16-09-1987
		JP 62502618 T		08-10-1987
		WO 8606384 A		06-11-1986
<hr/>				
WO 9732862 A	12-09-1997	IT M1968458 A		08-09-1997
		AU 2094697 A		22-09-1997
		EP 0885202 A		23-12-1998
		JP 2000506152 T		23-05-2000
<hr/>				

For more details about this annex: see Official Journal of the European Patent Office, No. 12/82

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER: _____**

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.